NIH Public Access
Author Manuscript
Expert Opin Biol Ther. Author manuscript; available in PMC 2010 February 10.

Published in final edited form as:
Expert Opin Biol Ther. 2007 February ; 7(2): 197. doi:10.1517/14712598.7.2.197.

The status of gene therapy for brain tumors

Giulia Fulci, PhD[1,2] and **E Antonio Chiocca, MD, PhD**[2]

Giulia Fulci: gfulci@partners.org; E Antonio Chiocca:

[1] Brain Tumor Research Center, Simches Research Building CRPZN-3800, Neurosurgery Service, Massachusetts General Hospital, 185 Cambridge Street, Boston, MA 02114, USA, Tel: +1 617 643 3431; Fax: +1 617 643 3422

[2] Dardinger Center for Neuro-oncology and Neurosciences, Department of Neurological Surgery, Arthur G. James Cancer Hospital and Richard J. Solove Research Institute, Comprehensive Cancer Center, The Ohio State University Medical Center, Columbus, OH 43210, USA

Abstract

The advent of gene therapy in the early 1990's raised expectations for brain tumor therapies; however, whereas clinical trials in patients with malignant gliomas provided evidence of safety, therapeutic benefit was not convincing. These early forays resembled the historical introductions of other therapies that seemed promising, only to fail in human trials. Nevertheless, re-study in the laboratory and retesting in iterative laboratory–clinic processes enabled therapies with strong biological rationales to ultimately show evidence of success in humans and become accepted. Examples, such as organ transplantation, monoclonal antibody therapy and antiangiogenic therapy, provide solace that a strategy's initial lack of success in humans provides an opportunity for its further refinement in the laboratory and development of solutions that will translate into patient success stories. The authors herein summarize results from clinical trials of gene therapy for malignant gliomas, and discuss the influence of these results on present thought in preclinical research.

Keywords

brain tumors; clinical trials; gene therapy; preclinical research

1. Introduction

Gliomas, the most frequent brain tumors, affect ~ 15,000–18,000 individuals each year in the US, and, at present, average survival is ~ 14 months for patients harbouring the most malignant gliomas and treated with the standard therapy of surgery, radiation and chemotherapy [1,2]. Gliomas are seen as focal masses with infiltrating margins comprised of malignant cells that migrate towards normal brain tissue [3]. These migrating cells are difficult to target because they cannot be imaged, the blood–brain barrier (BBB) prevents systemic chemotherapies from reaching them and they resist usual treatments that select for dividing cells. The migration of these cells causes the tumors to recur soon after therapy, rendering existing treatment regimens only palliative [1,3].

Several modalities have been and continue to be tested to treat malignant gliomas [4–7], and gene therapy is undergoing extensive investigation [8–10] due to the widely characterized genetic defects of gliomas [1,11,12] and the delimited localization of these tumors within the brain.

Correspondence to: Giulia Fulci, gfulci@partners.org.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Gene therapy involves the delivery of genes that drive the therapy to the cancer cells. Thus, it is crucial to find a vector that delivers a specific gene to tumor tissue efficiently and to define the type of genes that kill tumor cells most effectively. Nevertheless, unexpected difficulties arising in clinical trials of gene therapy in patients with brain tumors have taken the research beyond exploration for the most efficient vector and therapeutic gene, and opened new fields of investigation to improve brain tumor therapies. Herein, the authors focus on these difficulties and how they are being addressed in experimental models.

2. Gene therapy systems

2.1 Gene therapy systems in preclinical studies

The vectors and genes tested in preclinical studies have been widely reviewed [8–10], and are mentioned only briefly here; readers are referred to previously published reviews for their more thorough discussion. The vectors can be subdivided into three major categories: cellular, non-viral and viral.

Cellular vectors include bacteria and progenitor cells; have both been tested relatively recently and differ very much from each other. Bacteria, such as *Bifidobacterium*, *Salmonella* and *Clostridium*, have been shown to be effective vehicles of cellular delivery, particularly to hypoxic regions of tumor, and to provide a potent immune response [13,14]. In clinical trials in patients with tumors outside the brain, the use of bacteria has been encouraging, but it will require further advances [15]. Neural stem or progenitor cells are migratory cells capable of self-renewal and differentiation to neurons and glial cells, and they have been shown to migrate towards cancer cells, thus representing a good means of targeting migrating gliomas [16,17]. Several attempts to insert cancer-therapeutic genes in these cells in preclinical studies have proved promising [16,18], but no clinical trials have been performed.

Non-viral vectors include naked DNA and liposomes, which are delivered through injection or tissue bombardment and enter the cancer cells by endocytosis [19]. Liposomes, advantageous as gene therapy vectors as they are easy to produce and preserve and offer low toxicity and immunogenicity [19], have been used in only one recent clinical trial for brain tumors with evidence of safety [20,21].

Viruses remain the most efficient vectors in transducing tumor cells and have been used in most gene therapy trials on brain tumors; they are subdivided into non-replicating viruses (NRVs) [22–28] and replication-competent/oncolytic viruses (OVs) [29–31]. NRVs include recombinant adenoviruses and retroviruses, infectious bacterial artificial chromosomes, herpes simplex virus (HSV) amplicons, nucleic acid-free lentiviral nanoparticles and adeno-associated viruses. NRVs are believed to be safer for therapy because they cannot replicate to form infectious progeny, and they have been used in several clinical trials for treatment of gliomas. OVs are believed to be more efficient as they can selectively replicate in cancer cells and form progeny that can spread throughout the tumor mass. OVs are strongly cytotoxic, even without carrying external genes, and their use without transgenes has been reported in clinical trials for brain tumors. However, laboratory studies have shown that inserting a therapeutic transgene can increase their therapeutic efficiency [29–31].

T lymphocytes, as well as NK and mesenchymal cells, were recently tested as a means to deliver OVs; some viruses, such as vaccinia, replicate slowly in these cells, thus enabling cell-mediated delivery to tumor before the virus lyses them [32–34].

For the above-mentioned vectors, the following four categories of therapeutic transgenes have been described in laboratory research and extensively reviewed [8–10,30,31,35–37]:

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

- transgenes that code for a tumor suppressor no longer expressed in cancer cells, for example, for *TP53* or for siRNA inhibiting overexpressed oncogenes, such as epidermal growth factor receptor
- transgenes that code for immunostimulatory molecules to establish an anticancer immune response (interleukin [IL]-2, -4, granulocyte-macrophage colony-stimulating factor, IL-12, etc.)
- transgenes that inhibit crucial physiological processes required for brain tumor development and progression, such as angiogenesis and invasion (e.g., soluble domains of the receptor for vascular endothelial growth factor)
- prodrug-activating transgenes that increase local cytotoxicity of anticancer drugs, for example, HSV *thymidine kinase* (*TK*) transgene in combination with systemically delivered ganciclovir

In addition, a new category of transgenes that allows imaging of gene therapy is being tested and is discussed below.

2.2 Clinical trials

Although several vectors for gene therapy are undergoing preclinical testing, few have undergone clinical trials: four NRV systems (retrovirus carrying the *HSV-TK* gene or the *HSV-TK/IL2* cassette and adenovirus engineered with either *TP53* or *HSV-TK*); four OVs (HSV-derived G207 and 1716, adenovirus-derived ONYX-O15 and Newcastle disease virus [NDV]-HUJ); and non-viral liposomes carrying the *HSV-TK* gene. Tables 1 and 2 summarize the results of 20 clinical trials published to date [20,38–56].

The first attempt to use non-replicating retrovirus as a vector to deliver the *HSV-TK* gene to a patient's glioma was published in 1996 by Izquierdo's group [38], who demonstrated the safety and feasibility of a single intratumoral injection of retrovirus-producing cells (RVPC) followed by intravascular (intravenous or intra-arterial) delivery of ganciclovir. Since then, several clinical trials have evaluated the safety and efficacy of this therapeutic strategy [39–45]. The vector was delivered through multiple RVPC injections guided by gadolinium-enhanced magnetic resonance imaging (MRI) throughout the bed of resected tumors. Toxicity was evaluated by clinical adverse events and local inflammatory reactions, and efficacy was determined by MRI evaluation of tumor regression/progression and survival. Infiltration of immune cells and inflammatory reactions that occurred did not cause toxicity or treatment-related adverse events, demonstrating the safety of the strategy. Nevertheless, the efficacy of the treatment was unproved, and positive response to therapy was observed only in isolated events represented by patients with small tumors [39–41,45]. Ultimately, a randomized, controlled trial in 248 patients demonstrated no significant difference between therapeutic response to gene therapy with retrovirus carrying *HSV-TK* gene compared with standard radiation therapy [45]. This was explained by the difficulty to deliver ganciclovir through the BBB, inefficient intratumoral spread of RVPC and low retroviral transduction of tumor cells.

Indeed, although most trials have confirmed the viability of RVPC and the presence of *HSV-TK* within tumor biopsies, Harsh *et al.* [43] assessed a double-injection strategy that allowed immunohistochemical evaluation of the distribution of RVPC and *HSV-TK*. They concluded that *HSV-TK* was present only in RVPC and distributed no further than 10 mm from the injection site, indicating that any therapeutic result would be provided only by the bystander activity of the thymidine kinase [43]. The virus-mediated infiltration of immune cells led to the development of a retroviral vector carrying the *HSV-TK* and *IL2* genes to combine TK/ganciclovir chemo- with immunotherapy, but therapeutic efficacy did not appear to be improved [46,47].

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Studies performed in Finland have been more encouraging. One trial, completed in 10 patients, evaluated the extent of glioma transduction after injection of RVPC-*LacZ* versus adenovirus-*LacZ* [57], and determined glioma *LacZ* transduction to be between 0.01 and 4% when using RVPC, and between 0.01 and 11% with adenovirus [57]. Biologically, this can be justified by the higher titers that can be generated with adenoviral vectors and because adenoviral transduction does not require active cell division as does retroviral-mediated transduction. At any one time, only a small percentage of glioma cells are mitotic and, thus, targets for retroviral-mediated transduction, but adenoviral-mediated transduction can occur in both mitotic and non-mitotic glioma cells. In a second trial, the Finnish researchers compared survival of human patients randomized with treatment with RVPC-*HSV-TK* plus ganciclovir with that of patients treated with adenovirus-*HSV-TK* plus ganciclovir, and they determined that mean survival was < 9 months in the former group, but ≥ 15 months in the latter [48]. Although the groups were small, these findings encouraged a randomized trial comparing adenovirus-*HSV-TK* plus ganciclovir with standard treatment [52], which showed significantly prolonged survival in the former group. Thus, exploration of the use of adenovirus to deliver *HSV-TK* in combination with administration of ganciclovir or other nucleoside analogs as a therapeutic alternative may be warranted [49,50,52]. One report determined that the transduction efficiency of an adenovirus vector engineered with the *TP53* tumor suppressor gene was limited to an area of tumor averaging ~ 5 mm from the injection track [51]. However, additional results show that combining TK delivery by adenovirus with radiation therapy can synergistically produce both an anticancer pharmacologic effect and an immune effect [58]. To test this hypothesis, a Phase I trial is underway that combines gene with radiation therapy (EAC, unpublished).

The limited intratumoral distribution of NRVs tested for gene therapy led to trials using OVs, and although such use carried a potential risk of high toxicity from their replication capacity, the OVs were expected to spread further from the injection track and infect a larger tumor area than NRVs. The virus strains tested were HSV-derived G207 and 1716 [53,54], adenovirus-derived ONYX-O15 [55], and NDV [56]. To increase viral distribution within tumors, NDV was delivered intravenously. None of the trials reached the maximum tolerated dose (MTD) of virus, suggesting safety of the strategy; however, evidence of efficacy was still lacking. Finally, a recent clinical trial established the feasibility and safety of convection-enhanced delivery (CED) of liposomes carrying the *HSV-TK* gene, but the therapeutic efficiency remained low [20]. This trial is important because its testing of new delivery and imaging techniques led to a more detailed understanding of tumor response to gene therapy. It is possible that CED will provide a local delivery modality for NRVs and OVs that could result in a larger volume of distribution.

Thus, therapeutic inefficacy in clinical trials has most likely resulted from suboptimal tumor transduction of the vectors used. Transduction inefficiency could result from a variety of factors, related to both the brain tumor physiology and the vector's characteristics, alone or in combination due to: insufficient viral titers; mechanical difficulties in the process of vector delivery; insufficient dispersal of vector throughout the tumor matrix; host defense responses against the vector and its transduced genes; tumor heterogeneity; inefficient replication of OV in tumor cells; and acquired resistance of tumor cells to the therapy. A more detailed understanding of the aforementioned limitations will improve therapeutic success and can be achieved by establishing *in vivo* imaging methods that can follow the persistence and intratumoral spread of the vectors and/or their therapeutic genes, tumor response to therapy and intracerebral initiation of host defense responses over time.

The authors succinctly review the pertinent and most recent literature on each of the above limitations and provide opinions regarding where advances may occur.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

3. Limitations for successful outcome of brain tumor gene therapy

3.1 Insufficient viral titers

Only one trial of gene therapy for gliomas has established a MTD in humans. This strongly suggests that administered titers could have been and may remain below a possible effective dose. The only published human trial to reach a toxic dose showed that a single stereotactic injection of 2×10^{12} viral particles (vp) of adenovirus-*HSV-TK* into two patients with recurrent malignant gliomas was associated with edema and altered consciousness, establishing the toxicity of this dose for this particular construct in this type of disease (i.e., unresected recurrent malignant glioma treated with a single injection) [49]. Based on this finding, the MTD of adenoviral vectors seems to be $< 10^{12}$ vp for unresected gliomas, and a trial for newly diagnosed gliomas (resected and unresected) has been treating patients at this dose. Improved production methods to maximize vector titers will be important to fully exploit Phase I trials and find the MTD so that more detailed efficacy (Phase II and III) trials can be conducted with doses that are non-toxic, but potentially maximally effective. For example, novel production methods for a vector that is notoriously difficult to grow at high titers may provide avenues to resolve this limitation [59,60].

3.2 Mechanical viral delivery techniques

Most commonly, vectors are delivered by direct injection of the virus into the tumor bed following surgical tumor removal, but interstitial pressure from the skull created by the presence of tumor and normal tissue within the brain limits the intracerebral distribution of vectors. To increase the area of intratumoral viral spread, up to 70 virus injections in different sites have been performed [52]. Another way to increase intratumoral spread of the virus is to apply a pressure gradient in the extracellular space to create a convection flow (CED), which results in the distal diffusion of the injected substance through the tumor tissue [61]. The outcome of this delivery technique depends mainly on the chemical and physical characteristics of the injected substance [62], and has been shown to be possible for liposomes and viruses [61,63]. Nevertheless, delivery of liposomes carrying the *HSV-TK* gene by CED was not therapeutically advantageous in clinical trials [20], probably due to the limited capacity of liposomes to penetrate tumor cells. The problems to be solved with CED will relate to the stability of vector preparations in syringes/pumps at the patient's bedside, issues of safety to nursing and other personnel with CED delivery systems containing gene therapy at the bedside, and the possibility that vectors may bind to tubing.

Intraventricular injection was thought to be an alternative as it is relatively non-invasive and circulating cerebrospinal fluid might facilitate viral distribution, but animal studies showed high toxicity from inflammatory responses [64,65]. On the other hand, intravascular (intravenous and intra-arterial) delivery has proven safe in animal models and allows targeting of regions of tumor neovascularization, which represent migrating areas of gliomas; however, inhibition of viral trespassing through the BBB, neutralization of the virus by innate and pre-existing host immunity [66,67], and engulfment of vp in the liver limit the efficacy of intravascular (intravenous or intra-arterial) delivery. Bench research has shown that the BBB may be circumvented using osmotic and pharmacologic agents that increase trespassing of adenovirus and HSV [68,69]; selective intra-arterial instead of intravenous delivery may circumvent the first-pass effect of liver engulfment of vp [70,71]; and complement activation against the injected vector was inhibited using immunomodulation [72–74].

A recently published clinical trial [56] showed that intravenous delivery of NDV was possible in humans with glioma, but, clearly, additional advances will be needed.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

3.3 Insufficient dispersal through the matrix

One limitation to efficient viral intratumoral spread may be the compact network formed by the extracellular matrix. To overcome this, pretreatment with proteases was tested in experimental models of gliomas and melanomas, and was shown to be capable of increasing cancer cell transduction by NRV adenovirus and distal intratumoral spread of HSV OV, correlating with longer animal survival [75,76].

3.4 Host immune responses

Most viral vectors tested in clinical trials have been derived from viruses endemic to humans, and intravascular delivery of such viruses will probably be neutralized by antibodies, such as those against HSV and adenovirus [66,67]. However, evidence is lacking that such neutralization could limit clinical outcome of present human clinical trials that deliver OVs directly into the tumor bed. The presence within the tumor stroma of cells of the innate immune system, which rapidly react to the vectors used for gene therapy, may limit the outcome of this therapeutic strategy even when vectors are delivered directly to the tumor site [77,78], and infiltration of immune cells was observed in almost all clinical trials published. The authors have shown that intratumoral delivery of OVs to several models of gliomas in both mice and rats pretreated with the immunomodulating agent cyclophosphamide increases > 10-fold the capacity of viral intratumoral spread and replication, which increases survival [79–81]. It has been shown to be attributable to cyclophosphamide inhibition of OV elicitation of antiviral innate immune responses mediated by microglia/macrophages and natural killer cells, which correlates with a rapid decrease of viral persistence in the tumor [80,81]. It is unclear whether these innate immune responses derive from the periphery or are mediated primarily by intracerebral defense mechanisms. This knowledge is important both to evaluate whether the role of pre-existing immunity to the vector should be a concern when the vector is delivered *in situ*, and to design immunomodulating strategies that can improve the therapeutic outcome of gene therapy.

3.5 Tumor heterogeneity

Glioblastoma multiforme, the most malignant and frequent brain tumor, is thus named for its heterogeneity. Efficient treatment is difficult when targeting specific genetic mutations due to the various genetic alterations affecting the neoplastic cells that form this tumor. Moreover, due to the heterogeneous presentation of receptors for vectors used in gene therapy, not all tumor cells are susceptible to vector interaction with the same efficacy.

Studies by Curiel and colleagues [82] have elucidated the requirements for Coxsackie and adenovirus receptor (CAR) expression in cells to achieve efficient infection of cells, and have discovered that CAR is not highly expressed in a number of tumors, including gliomas. This finding has spawned interest in re-engineering adenoviruses with tropism that is redirected away from CAR toward receptors that are overexpressed in gliomas, such as integrins [83]. These RGD-modified adenoviruses display increased infectivity for gliomas and, thus, suggest a potential avenue for increased efficacy. HSV1 tropism may also depend on expression of HveC on tumor cell surfaces, so tropism redirected toward molecules expressed on glioma surfaces is also possible for this virus [84].

3.6 Inefficient replication of oncolytic viruses

The efficacy of OVs against tumors is predicated on the principle that greater viral replication (i.e., virion progeny production) results in increased infection and death of tumor cells, so attenuation by genetic means may impair robust replication. As an example, replication of HSV1 mutants in *ICP34.5* gene function (the most common virus tested clinically) may be impaired, but such impairment has been circumvented using either tumor-specific promoters

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

to drive expression of *ICP34.5* [85–89] or second-site suppressor mutations [90,91]. Several tumor-specific promoters have also been used to drive expression of adenoviral genes [92].

3.7 Establishing resistance

The exciting discovery that viral mutants can replicate in tumor cells because a tumor suppressor gene pathway is absent led to the construction of replicating viruses that target tumors [93–96]; however, this targeted selectivity may also generate tumor cells within a neoplasm that resist treatment by escaping from the particular targeted pathway. An avenue to circumvent such a limitation may be in the generation of viruses by serial culture in tumor cells to find viruses with multiple mutations that target multiple pathways needed for tumorigenesis [97].

Another mechanism to circumvent tumor cell resistance will be combining therapies. As an example, HSV has been engineered to express chemotherapy-activating gene [98–100] and has worked synergistically with temozolomide, a standard chemotherapeutic, in oncolysis [101].

4. Means and models for experimental therapeutics

4.1 Animal models for glioma experimental therapeutics

One major difficulty in establishing treatments for malignant gliomas is the absence of an efficient animal model for preclinical studies. Commonly, glioma cells are injected into the brains or flanks of animals. Such a system is practical for experimental therapeutics, but it does not accurately reproduce the real features of malignant gliomas. These tumors will comprise cells that have all the same genetic alterations and interact with gene therapy vectors with the same efficacy as real tumors, are well circumscribed and usually do not invade the parenchyma to the same extent as normal gliomas, and are very close to each other, thereby enhancing the bystander effect of gene products used in gene therapy. In addition, primary human tumor xenografts have been established in immunocompromised animals to reproduce the genetic diversity of human gliomas, but these animal models do not realistically represent the host–tumor interaction [102]. Thus, an effort is being made to develop animal models that replicate glioma physiology in immunocompetent animals, such as germ line genetically engineered models [103,104], somatic cell gene transfer through retroviral vectors [105] or embryonic stem cells transgenesis [106]. Nonetheless, the use of such models for preclinical brain tumor therapy is very limited as detecting and visualizing the tumor is difficult and the waiting time to obtain a tumor is long. Moreover, results from the use of all animal models available for evaluating experimental therapeutics for brain tumors are biased by the small size of the tumors, which facilitate delivery of therapeutic substances. In conclusion, despite preclinical research efforts, the absence of a good animal model makes translation of therapeutic efficacy from rodents to humans difficult, so that therapies efficient in rodents fail in humans.

4.2 Imaging

In most trials reported, response to therapy was assessed not only by survival, but also by tumor regression or progression, as established through gadolinium-enhanced MRI. This non-invasive imaging of hypercellular areas with neovascularization provides information on tumor vascularity, size and location [107]. Performed at different time points throughout treatment, such imaging elucidates the difficulties associated with gene therapy by revealing small changes occurring in tumor volume over time; therefore, improving such non-invasive imaging techniques will enhance the quality and quantity of information about the disease condition and efficacy of the therapeutic modality employed.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Positron emission tomography (PET), a more novel molecular imaging technique, allows quantification of the tumor's metabolic state [108–110]. PET enables visualization of several cellular functions related to increased proliferation, such as increased activity of membrane transporters and cellular hexokinase and thymidine kinase [108–110], to allow differentiation of normal brain tissue, low-grade glioma, high-grade glioma and necrotic tissue. Information provided by PET complements data from MRI regarding certain tumoral aspects [108–110]. MRI and PET can be used during gene therapy to guide tumor resection, viral injection and stereotactic biopsy of tumor specimens, and to quantify tumor response to therapy. The capacity of PET to image molecular activity, such as membrane transporter and TK, also permits visualization of intratumoral distribution of transgenes tested for gene therapy, such as *sodium^{+}/iodide^{-}transporter* and *HSV-TK* [111–113]. In one clinical trial, in which *HSV-TK* transgene was delivered by liposome vector using CED injection, MRI and PET provided detailed quantification of tumor response to gene therapy versus CED-mediated distribution of the vector and *HSV-TK* gene expression in cancer cells [20]. The results indicate a correlated vector distribution and tumor response, and suggest the heterogeneity of glioma tissues as the main limit to successful therapy.

The teams of Schellingerhout [71] and Rehemtulla [114] have employed molecular imaging in experimental glioma models in which vectors radiolabelled with ^{111}In-Oxine or engineered with an *Escherichia coli LacZ* gene were delivered. Both groups quantified a precise mass distribution of the vector [71,114]. Another group also used an OV adenovirus engineered with the *E. coli LacZ* gene to quantify and compare intratumoral replication and persistence of the OV in the presence and absence of the immunomodulator cyclophosphamide, thus demonstrating the possibility of non-invasive *in vivo* imaging of the efficiency of gene therapy applied in combination with other chemotherapeutic agents [81].

Finally, analysis of single photon computed tomography performed with the 3-[123]iodo-L-alpha-methyl-tyrosine ([^{123}I]IMT) on 11 patients from multicenter clinical trials has shown a 'flare' enhancement that correlated with strong inflammatory reaction [115]. Inflammatory reactions are common side effects of brain tumor treatment, in particular, gene therapy, and their precise influence on therapy outcome has not been determined. Indeed, it is unclear whether these inflammatory reactions are important in establishing antitumoral immune response and providing a therapeutic advantage, if they inhibit intratumoral persistence of virus and limit therapeutic outcome, or if they induce stromal changes that assist tumor recurrence by increasing angiogenesis and migration of cancer cells. Thus, developing imaging techniques that provide quantitative and temporal information about inflammatory reactions during gene therapy would help clarify how immune stimulation mediated by gene therapy interferes with persistence of intratumoral vector or helps tumor therapy by establishing an antitumoral immune response. Moreover, such techniques could elucidate the stromal changes in tumor induced by these inflammatory responses.

Recently, a molecular imaging technique based on MRI of tumor-infiltrating macrophages that employs dextran-coated monocrystalline iron oxide particles has been used. Phagocytosed by circulating macrophages, the monocrystalline iron oxide particles give the macrophages superparamagnetic properties that enable MRI without affecting macrophage function [80]. This technique has been used to quantify OV-mediated tumor infiltration of peripheral macrophages in an experimental rat glioma model, and found that pretreatment of the animals with cyclophosphamide inhibits this infiltration and that the infiltration correlates with increased intratumoral replication and persistence of virus [80].

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

5. Conclusions

The need to further develop gene therapies for malignant brain tumors is clear from the scarcity of effective treatments as well as the promise emanating from the elucidation of the genetic and physical features of such tumors and the positive outcomes reported in experimental models of gene therapy. Clinical trials performed to date have proven that this therapeutic technique is feasible and relatively safe, but have not yielded advanced therapeutic results. Great efforts have been invested in finding the best system for gene therapy of brain tumors and have included investigation of several vector systems and transgenic genes. The development of viruses that selectively replicate in cancer cells has further broadened the hopes for this therapeutic strategy. Indeed, OVs have the potential to lyse cancer cells and to carry one or more transgenes that could increase antitumoral immunogenicity, increase intratumoral concentration of a prodrug, or buffer the genetic alterations typical of brain tumor development and progression. Thus, OVs could be used in multimodal therapies. Nevertheless, a lack of understanding of the mechanism of efficient intratumoral vector delivery continues to block efficient therapeutic outcome. Research on experimental models of glioma suggests that better characterization of the tumor stroma and of the host physiological responses to viral delivery may be key to solving the problems related to virus delivery, and developing non-invasive molecular imaging techniques is essential to evaluate the progress following virus delivery.

6. Expert opinion

Over the last 10 years, various gene therapy systems have been tested in several clinical trials in patients with malignant gliomas (Tables 1 and 2), and the results of all of the trials published conclude that: gene therapy for malignant gliomas is safe and feasible, but inefficient at present; and inefficient delivery of the therapeutic vectors and transgenes represents the major limit for successful outcome of this therapeutic strategy. The results from these trials do not differ significantly from the results obtained from standard postsurgical debulking chemo- and radiotherapies, and survival rates are very similar. Investigators are diligently working towards achieving successful performance of OV *in vivo*. Due to the capacity of these viruses to replicate within cancer cells and spread their progeny throughout the tumor, they have been perceived as having great potential for solving the problem of intratumoral distribution of drug. However, animal models and clinical trials using OVs in patients with malignant gliomas seem to show that OVs do not replicate as efficiently *in vivo* as *in vitro*. Nevertheless, comparison of the poor intratumoral distribution of virus with therapeutic outcome indicates that gene therapy using an improved vector has strong potential in the treatment of brain tumors. Moreover, the development of gene therapy for brain tumors has brought to light two fundamental novelties for the treatment of brain tumors: the evolution of molecular imaging techniques to evaluate the physiological and molecular consequences of the therapy; and clarification of the inflammatory processes that underlie treatment of brain tumors. The potential to image the intratumoral distribution of the vector by detection of transgenes non-invasively by using PET has led to strong efforts to develop molecular imaging techniques. Today, non-invasive imaging in clinical trials can quantify the regression/progression and metabolic and stromal characteristics of tumor, intratumoral distribution of the vector, and persistence and appearance of inflammatory reactions in a time-dependent manner. An understanding of all these data underlies a comprehension of the limits of brain tumor therapies and the need to develop new means to efficiently target cancer cells disseminated through the brain using therapeutic drugs.

Gene therapy has also served to shift our focus towards the problem of inflammatory reactions from brain tumor treatment. Although inflammation also arises with standard radio- and chemotherapy, it has never been understood as a means to alter tumor therapeutics as host immune responses are unlikely to limit the intratumoral penetration of radiation. However, the

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

problem of a host immune system that limits the capacity of viral vectors to perform their therapeutic duties has raised concern regarding the significance of such inflammatory responses to brain tumor progression and treatment. Indeed, glial cells in the brain react very rapidly and efficiently to exogenous substances to produce inflammatory responses that provide chemoresistance [116], immunosuppression [117] and tumorigenic changes of the stroma [118].

Finally, experimental models of malignant gliomas have demonstrated the potential use of OVs in multimodal therapies. Combination therapy helps address the problem of tumor diversity. OVs can kill cancer cells as well as carrying genes that enhance a chemotherapeutic response, inhibit angiogenesis or activate antitumor immunity, but multimodal therapies using OVs have not yet been tested in clinical trials. Therefore, the authors believe that the testing of gene therapy for brain tumors is only beginning and that the amount of novel information generated using this therapeutic strategy should support further research in this field.

Acknowledgments

The authors acknowledge the following funding: P01- MGH parent grant #CA69246–10A1- NIH/NCI and F Hochberg (Director), EAC (PI of project 1).

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1•. LOUIS DN, POMEROY SL, CAIRNCROSS JG. Focus on central nervous system neoplasia. Cancer Cell 2002;1(2):125–128. Comprehensive review on brain tumors that includes all their histological features relevant to design treatments. [PubMed: 12086870]

2••. STUPP R, MASON WP, VAN DEN BENT MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 2005;352:10–996. First clinical trial showing significant improvement in the treatment of malignant gliomas.

3. KLEIHUES, P.; CAVENEE, WK. Pathology and Genetics of Tumors of the Nervous System. Kleihues, P.; Cavenee, WK., editors. International Agency for Research on Cancer; Lyon, France: 1997.

4. KIRSCH M, SCHACKERT G, BLACK PM. Anti-angiogenic treatment strategies for malignant brain tumors. J Neurooncol 2000;50(1–2):149–163. [PubMed: 11245274]

5. JENDROSSEK V, BELKA C, BAMBERG M. Novel chemotherapeutic agents for the treatment of glioblastoma multiforme. Expert Opin Investig Drugs 2003;12(12):1899–1924.

6. EHTESHAM M, BLACK KL, YU JS. Recent progress in immunotherapy for malignant glioma: treatment strategies and results from clinical trials. Cancer Control 2004;11(3):192–207. [PubMed: 15153843]

7. GUERIN C, OLIVI A, WEINGART JD, LAWSON HC, BREM H. Recent advances in brain tumor therapy: local intracerebral drug delivery by polymers. Invest New Drugs 2004;22(1):27–37. [PubMed: 14707492]

8. LAM PY, BREAKEFIELD XO. Potential of gene therapy for brain tumors. Hum Mol Genet 2001;10 (7):777–787. [PubMed: 11257112]

9. CHIOCCA EA, AGHI M, FULCI G. Viral therapy for glioblastoma. Cancer J 2003;9(3):167–179. [PubMed: 12952302]

10. AGHI M, RABKIN S. Viral vectors as therapeutic agents for glioblastoma. Curr Opin Mol Ther 2005;7(5):419–430. [PubMed: 16248277]

11. ICHIMURA K, BOLIN MB, GOIKE HM, SCHMIDT EE, MOSHREF A, COLLINS VP. Deregulation of the p14ARF/MDM2/p53 pathway is a prerequisite for human astrocytic gliomas with G1-S transition control gene abnormalities. Cancer Res 2000;60(2):417–424. [PubMed: 10667596]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

12. FULCI G, LABUHN M, MAIER D, et al. p53 gene mutation and ink4a-arf deletion appear to be two mutually exclusive events in human glioblastoma. Oncogene 2000;19(33):3816–3822. [PubMed: 10949938]

13. YAZAWA K, FUJIMORI M, AMANO J, et al. Bifidobacterium longum as a delivery system for cancer gene therapy: selective localization and growth in hypoxic tumors. Cancer Gene Ther 2000;7 (2):269–274. [PubMed: 10770636]

14. AGRAWAL N, BETTEGOWDA C, CHEONG I, et al. Bacteriolytic therapy can generate a potent immune response against experimental tumors. Proc Natl Acad Sci USA 2004;101(42):15172–15177. [PubMed: 15471990]

15. VASSAUX G, NITCHEU J, JEZZARD S, LEMOINE NR. Bacterial gene therapy strategies. J Pathol 2006;208(2):290–298. [PubMed: 16362987]

16. ABOODY KS, BROWN A, RAINOV NG, et al. Neural stem cells display extensive tropism for pathology in adult brain: evidence from intracranial gliomas. Proc Natl Acad Sci USA 2000;97(23): 12846–12851. [PubMed: 11070094]

17. GLASS R, SYNOWITZ M, KRONENBERG G, et al. Glioblastoma-induced attraction of endogenous neural precursor cells is associated with improved survival. J Neurosci 2005;25(10):2637–2646. [PubMed: 15758174]

18. BENEDETTI S, PIROLA B, POLLO B, et al. Gene therapy of experimental brain tumors using neural progenitor cells. Nat Med 2000;6(4):447–450. [PubMed: 10742153]

19. YOSHIDA J, MIZUNO M. Clinical gene therapy for brain tumors. Liposomal delivery of anticancer molecule to glioma. J Neurooncol 2003;65(3):261–267. [PubMed: 14682376]

20•. VOGES J, RESZKA R, GOSSMANN A, et al. Imaging-guided convection-enhanced delivery and gene therapy of glioblastoma. Ann Neurol 2003;54(4):479–487. First clinical trial using a non-viral gene therapy vector in combination with novel imaging and delivery techniques; this trial provides important information about existing gene therapy limits. [PubMed: 14520660]

21. MIZUNO M, YOSHIDA J. Gene therapy for malignant glioma. Nippon Rinsho 2005;63(3):469–475. [PubMed: 15773347]

22. RAINOV NG, REN H. Clinical trials with retrovirus mediated gene therapy-what have we learned? J Neurooncol 2003;65(3):227–236. [PubMed: 14682373]

23. VECIL GG, LANG FF. Clinical trials of adenoviruses in brain tumors: a review of Ad-p53 and oncolytic adenoviruses. J Neurooncol 2003;65(3):237–246. [PubMed: 14682374]

24. INOUE R, MOGHADDAM KA, RANASINGHE M, SAEKI Y, CHIOCCA EA, WADE-MARTINS R. Infectious delivery of the 132 kb CDKN2A/CDKN2B genomic DNA region results in correctly spliced gene expression and growth suppression in glioma cells. Gene Ther 2004;11(15):1195–1204. [PubMed: 15164098]

25. REINBLATT M, PIN RH, BOWERS WJ, FEDEROFF HJ, FONG Y. Herpes simplex virus amplicon delivery of a hypoxia-inducible soluble vascular endothelial growth factor receptor (sFlk-1) inhibits angiogenesis and tumor growth in pancreatic adenocarcinoma. Ann Surg Oncol 2005;12(12):1025–1036. [PubMed: 16244806]

26. SAYDAM O, GLAUSER DL, HEID I, et al. Herpes simplex virus 1 amplicon vector-mediated siRNA targeting epidermal growth factor receptor inhibits growth of human glioma cells *in vivo*. Mol Ther 2005;12(5):803–812. [PubMed: 16112910]

27. LI C, BOWLES DE, VAN DYKE T, SAMULSKI RJ. Adeno-associated virus vectors: potential applications for cancer gene therapy. Cancer Gene Ther 2005;12(12):913–925. [PubMed: 15962012]

28. LINK N, AUBEL C, KELM JM, et al. Therapeutic protein transduction of mammalian cells and mice by nucleic acid-free lentiviral nanoparticles. Nucleic Acids Res 2006;34(2):e16. [PubMed: 16449199]

29. KIRN D, MARTUZA RL, ZWIEBEL J. Replication-selective virotherapy for cancer: biological principles, risk management and future directions. Nat Med 2001;7(7):781–787. [PubMed: 11433341]

30. FULCI G, CHIOCCA EA. Oncolytic viruses for the therapy of brain tumors and other solid malignancies: a review. Front Biosci 2003;8:e346–e360. [PubMed: 12700108]

31. AGHI M, MARTUZA RL. Oncolytic viral therapies – the clinical experience. Oncogene 2005;24 (52):7802–7816. [PubMed: 16299539]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

32. COLE C, QIAO J, KOTTKE T, et al. Tumor-targeted, systemic delivery of therapeutic viral vectors using hitchhiking on antigen-specific T cells. Nat Med 2005;11(10):1073–1081. [PubMed: 16170322]
33. KOMAROVA S, KAWAKAMI Y, STOFF-KHALILI MA, CURIEL DT, PEREBOEVA L. Mesenchymal progenitor cells as cellular vehicles for delivery of oncolytic adenoviruses. Mol Cancer Ther 2006;5(3):755–766. [PubMed: 16546991]
34. THORNE SH, NEGRIN RS, CONTAG CH. Synergistic antitumor effects of immune cell-viral biotherapy. Science 2006;311(5768):1780–1784. [PubMed: 16556847]
35. POST DE, FULCI G, CHIOCCA EA, VAN MEIR EG. Replicative oncolytic herpes simplex viruses in combination cancer therapies. Curr Gene Ther 2004;4(1):41–51. [PubMed: 15032613]
36. DONG JY, WORARATANADHARM J. Gene therapy vector design strategies for the treatment of cancer. Future Oncol 2005;1(3):361–373. [PubMed: 16556010]
37. LAGE H. Potential applications of RNA interference technology in the treatment of cancer. Future Oncol 2005;1(1):103–113. [PubMed: 16555980]
38. IZQUIERDO M, MARTIN V, DE FELIPE P, et al. Human malignant brain tumor response to herpes simplex thymidine kinase (HSVtk)/ganciclovir gene therapy. Gene Ther 1996;3(6):491–495. [PubMed: 8789798]
39. RAM Z, CULVER KW, OSHIRO EM, et al. Therapy of malignant brain tumors by intratumoral implantation of retroviral vector-producing cells. Nat Med 1997;3(12):1354–1361. [PubMed: 9396605]
40. KLATZMANN D, VALERY CA, BENSIMON G, et al. A Phase I/II study of herpes simplex virus type 1 thymidine kinase 'suicide' gene therapy for recurrent glioblastoma. Study Group on Gene Therapy for Glioblastoma. Hum Gene Ther 1998;9(17):2595–2604. [PubMed: 9853526]
41. SHAND N, WEBER F, MARIANI L, et al. A Phase I-2 clinical trial of gene therapy for recurrent glioblastoma multiforme by tumor transduction with the herpes simplex thymidine kinase gene followed by ganciclovir. GLI328 European-Canadian Study Group. Hum Gene Ther 1999;10(14): 2325–2335. [PubMed: 10515452]
42. ADACHI N, KONU DL, FREI K, YONEKAWA Y. The HSV-TK/GCV gene therapy in five cases of recurrent glioblastoma multiforme. No Shinkei Geka 2000;28(10):865–871. [PubMed: 11070906]
43. HARSH GR, DEISBOECK TS, LOUIS DN, et al. Thymidine kinase activation of ganciclovir in recurrent malignant gliomas: a gene-marking and neuropathological study. J Neurosurg 2000;92(5): 804–811. [PubMed: 10794295]
44. PACKER RJ, RAFFEL C, VILLABLANCA JG, et al. Treatment of progressive or recurrent pediatric malignant supratentorial brain tumors with herpes simplex virus thymidine kinase gene vector-producer cells followed by intravenous ganciclovir administration. J Neurosurg 2000;92(2):249–254. [PubMed: 10659011]
45••. RAINOV NG. A Phase III clinical evaluation of herpes simplex virus type 1 thymidine kinase and ganciclovir gene therapy as an adjuvant to surgical resection and radiation in adults with previously untreated glioblastoma multiforme. Hum Gene Ther 2000;11(17):2389–2401. Clinical trial that demonstrated the lack of improvement in brain tumor therapy when using retrovirus gene therapy systems; this study has pushed clinicians and researchers to study new vector systems for gene therapy. [PubMed: 11096443]
46. PALU G, CAVAGGIONI A, CALVI P, et al. Gene therapy of glioblastoma multiforme via combined expression of suicide and cytokine genes: a pilot study in humans. Gene Ther 1999;6(3):330–337. [PubMed: 10435083]
47. COLOMBO F, BARZON L, FRANCHIN E, et al. Combined HSV-TK/IL-2 gene therapy in patients with recurrent glioblastoma multiforme: biological and clinical results. Cancer Gene Ther 2005;12 (10):835–848. [PubMed: 15891772]
48. SANDMAIR AM, LOIMAS S, PURANEN P, et al. Thymidine kinase gene therapy for human malignant glioma, using replication-deficient retroviruses or adenoviruses. Hum Gene Ther 2000;11 (16):2197–2205. [PubMed: 11084677]
49. TRASK TW, TRASK RP, AGUILAR-CORDOVA E, et al. Phase I study of adenoviral delivery of the HSV-tk gene and ganciclovir administration in patients with current malignant brain tumors. Mol Ther 2000;1(2):195–203. [PubMed: 10933931]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

50. GERMANO IM, FABLE J, GULTEKIN SH, SILVERS A. Adenovirus/herpes simplex-thymidine kinase/ganciclovir complex: preliminary results of a Phase I trial in patients with recurrent malignant gliomas. J Neurooncol 2003;65(3):279–289. [PubMed: 14682378]
51. LANG FF, BRUNER JM, FULLER GN, et al. Phase I trial of adenovirus-mediated p53 gene therapy for recurrent glioma: biological and clinical results. J Clin Oncol 2003;21(13):2508–2518. [PubMed: 12839017]
52. IMMONEN A, VAPALAHTI M, TYYNELA K, et al. AdvHSV-tk gene therapy with intravenous ganciclovir improves survival in human malignant glioma: a randomised, controlled study. Mol Ther 2004;10(5):967–972. [PubMed: 15509514]
53. MARKERT JM, MEDLOCK MD, RABKIN SD, et al. Conditionally replicating herpes simplex virus mutant, G207 for the treatment of malignant glioma: results of a Phase I trial. Gene Ther 2000;7(10): 867–874. [PubMed: 10845725]
54. RAMPLING R, CRUICKSHANK G, PAPANASTASSIOU V, et al. Toxicity evaluation of replication-competent herpes simplex virus (ICP 34.5 null mutant 1716) in patients with recurrent malignant glioma. Gene Ther 2000;7(10):859–866. [PubMed: 10845724]
55. CHIOCCA EA, ABBED KM, TATTER S, et al. A Phase I open-label, dose-escalation, multi-institutional trial of injection with an E1B-attenuated adenovirus, ONYX-015, into the peritumoral region of recurrent malignant gliomas, in the adjuvant setting. Mol Ther 2004;10(5):958–966. [PubMed: 15509513]
56. FREEMAN AI, ZAKAY-RONES Z, GOMORI JM, et al. Phase I/II trial of intravenous NDV-HUJ oncolytic virus in recurrent glioblastoma multiforme. Mol Ther 2006;13(1):221–228. [PubMed: 16257582]
57. PUUMALAINEN AM, VAPALAHTI M, AGRAWAL RS, et al. Beta-galactosidase gene transfer to human malignant glioma *in vivo* using replication-deficient retroviruses and adenoviruses. Hum Gene Ther 1998;9(12):1769–1774. [PubMed: 9721087]
58. NESTLER U, WAKIMOTO H, SILLER-LOPEZ F, et al. The combination of adenoviral HSV TK gene therapy and radiation is effective in athymic mouse glioblastoma xenografts without increasing toxic side effects. J Neurooncol 2004;67(1–2):177–188. [PubMed: 15072465]
59. JIANG C, WECHUCK JB, GOINS WF, et al. Immobilized cobalt affinity chromatography provides a novel, efficient method for herpes simplex virus type 1 gene vector purification. J Virol 2004;78 (17):8994–9006. [PubMed: 15308696]
60. KASAI K, SAEKI Y. DNA-based methods to prepare helper virus-free herpes amplicon vectors and versatile design of amplicon vector plasmids. Curr Gene Ther 2006;6(3):303–314. [PubMed: 16787182]
61. CHEN MY, HOFFER A, MORRISON PF, et al. Surface properties, more than size, limiting convective distribution of virus-sized particles and viruses in the central nervous system. J Neurosurg 2005;103(2):311–319. [PubMed: 16175862]
62. SAITO R, KRAUZE MT, NOBLE CO, et al. Tissue affinity of the infusate affects the distribution volume during convection-enhanced delivery into rodent brains: implications for local drug delivery. J Neurosci Methods 2006;154(1–2):225–232. [PubMed: 16472868]
63. SAITO R, KRAUZE MT, BRINGAS JR, et al. Gadolinium-loaded liposomes allow for real-time magnetic resonance imaging of convection-enhanced delivery in the primate brain. Exp Neurol 2005;196(2):381–389. [PubMed: 16197944]
64. OSHIRO EM, VIOLA JJ, OLDFIELD EH, et al. Toxicity studies and distribution dynamics of retroviral vectors following intrathecal administration of retroviral vector-producer cells. Cancer Gene Ther 1995;2(2):87–95. [PubMed: 7621261]
65. DRIESSE MJ, ESANDI MC, KROS JM, et al. Intra-CSF administered recombinant adenovirus causes an immune response-mediated toxicity. Gene Ther 2000;7(16):1401–1409. [PubMed: 10981667]
66. CHIRMULE N, PROPERT K, MAGOSIN S, QIAN Y, QIAN R, WILSON J. Immune responses to adenovirus and adeno-associated virus in humans. Gene Ther 1999;6(9):1574–1583. [PubMed: 10490767]
67. WAKIMOTO H, JOHNSON PR, KNIPE DM, CHIOCCA EA. Effects of innate immunity on herpes simplex virus and its ability to kill tumor cells. Gene Ther 2003;10(11):983–990. [PubMed: 12756419]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

68. NILAVER G, MULDOON LL, KROLL RA, et al. Delivery of herpesvirus and adenovirus to nude rat intracerebral tumors after osmotic blood-brain barrier disruption. Proc Natl Acad Sci USA 1995;92(21):9829–9833. [PubMed: 7568227]

69. BARNETT FH, RAINOV NG, IKEDA K, et al. Selective delivery of herpes virus vectors to experimental brain tumors using RMP-7. Cancer Gene Ther 1999;6(1):14–20. [PubMed: 10078959]

70. SCHELLINGERHOUT D, BOGDANOV A JR, MARECOS E, SPEAR M, BREAKEFIELD XO, WEISSLEDER R. Mapping the *in vivo* distribution of herpes simplex virions. Hum Gene Ther 1998;9 (11):1543–1549. [PubMed: 9694153]

71. SCHELLINGERHOUT D, RAINOV NG, BREAKEFIELD XO, WEISSLEDER R. Quantitation of HSV mass distribution in a rodent brain tumor model. Gene Ther 2000;7(19):1648–1655. [PubMed: 11083473]

72••. IKEDA K, ICHIKAWA T, WAKIMOTO H, et al. Oncolytic virus therapy of multiple tumors in the brain requires suppression of innate and elicited antiviral responses. Nat Med 1999;5(8):881–887. Preclinical study applying oncolytic viruses in combination with immunosuppressive chemotherapeutic agents; this study has showed for the first time that host innate immune responses may be detrimental for viral therapy, and presented a therapeutic strategy that strongly increases the efficacy of oncolytic viruses. [PubMed: 10426310]

73. IKEDA K, WAKIMOTO H, ICHIKAWA T, et al. Complement depletion facilitates the infection of multiple brain tumors by an intravascular, replication-conditional herpes simplex virus mutant. J Virol 2000;74(10):4765–4775. [PubMed: 10775615]

74. WAKIMOTO H, IKEDA K, ABE T, et al. The complement response against an oncolytic virus is species-specific in its activation pathways. Mol Ther 2002;5(3):275–282. [PubMed: 11863417]

75. KURIYAMA N, KURIYAMA H, JULIN CM, LAMBORN KR, ISRAEL MA. Protease pretreatment increases the efficacy of adenovirus-mediated gene therapy for the treatment of an experimental glioblastoma model. Cancer Res 2001;61(5):1805–1809. [PubMed: 11280727]

76. MCKEE TD, GRANDI P, MOK W, et al. Degradation of fibrillar collagen in a human melanoma xenograft improves the efficacy of an oncolytic herpes simplex virus vector. Cancer Res 2006;66(5): 2509–2513. [PubMed: 16510565]

77. LOWENSTEIN PR. The case for immunosuppression in clinical gene transfer. Mol Ther 2005;12 (2):185–186. [PubMed: 16002339]

78•. FRIEDMAN A, TIAN JP, FULCI G, CHIOCCA EA, WANG J. Glioma virotherapy: effects of innate immune suppression and increased viral replication capacity. Cancer Res 2006;66(4):2314–2319. A mathematical model describing the interaction between oncolytic viruses, the host immune system and immunosuppressive drugs; this model has predicted the outcome of preclinical research that has followed. [PubMed: 16489036]

79. WAKIMOTO H, FULCI G, TYMINSKI E, CHIOCCA EA. Altered expression of antiviral cytokine mRNAs associated with cyclophosphamide's enhancement of viral oncolysis. Gene Ther 2004;11 (2):214–223. [PubMed: 14712306]

80. FULCI G, BREYMANN L, GIANNI D, et al. Cyclophosphamide enhances glioma virotherapy by inhibiting innate immune responses. Proc Natl Acad Sci USA 2006;103(34):12873–12878. [PubMed: 16908838]

81. LAMFERS ML, FULCI G, GIANNI D, et al. Cyclophosphamide increases transgene expression mediated by an oncolytic adenovirus in glioma-bearing mice monitored by bioluminescence imaging. Mol Ther. 2006 (In Press).

82. DOUGLAS JT, KIM M, SUMEREL LA, CAREY DE, CURIEL DT. Efficient oncolysis by a replicating adenovirus (ad) *in vivo* is critically dependent on tumor expression of primary ad receptors. Cancer Res 2001;61(3):813–817. [PubMed: 11221860]

83. KASONO K, BLACKWELL JL, DOUGLAS JT, et al. Selective gene delivery to head and neck cancer cells via an integrin targeted adenoviral vector. Clin Cancer Res 1999;5(9):2571–2579. [PubMed: 10499635]

84. ZHOU G, ROIZMAN B. Characterization of a recombinant herpes simplex virus 1 designed to enter cells via the IL13Ralpha2 receptor of malignant glioma cells. J Virol 2005;79(9):5272–5277. [PubMed: 15827141]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

85. CHUNG R, SAEKI Y, CHIOCCA EA. B-myb promoter retargeting of herpes simplex virus gamma34.5 gene-mediated virulence toward tumor and cycling cells. J Virol 1999;73(9):7556–7564. [PubMed: 10438845]

86. NAKAMURA H, KASUYA H, MULLEN JT, et al. Regulation of herpes simplex virus gamma(1) 34.5 expression and oncolysis of diffuse liver metastases by Myb34.5. J Clin Invest 2002;109(7): 871–882. [PubMed: 11927614]

87. KASUYA H, PAWLIK TM, MULLEN JT, et al. Selectivity of an oncolytic herpes simplex virus for cells expressing the DF3/MUC1 antigen. Cancer Res 2004;64(7):2561–2567. [PubMed: 15059912]

88. KAMBARA H, OKANO H, CHIOCCA EA, SAEKI Y. An oncolytic HSV-1 mutant expressing ICP34.5 under control of a nestin promoter increases survival of animals even when symptomatic from a brain tumor. Cancer Res 2005;65(7):2832–2839. [PubMed: 15805284]

89. KANAI R, TOMITA H, SHINODA A, et al. Enhanced therapeutic efficacy of G207 for the treatment of glioma through Musashi1 promoter retargeting of gamma34.5-mediated virulence. Gene Ther 2006;13(2):106–116. [PubMed: 16163378]

90. MOHR I, STERNBERG D, WARD S, LEIB D, MULVEY M, GLUZMAN Y. A herpes simplex virus Type 1 gamma34.5 second-site suppressor mutant that exhibits enhanced growth in cultured glioblastoma cells is severely attenuated in animals. J Virol 2001;75(11):5189–5196. [PubMed: 11333900]

91•. TODO T, MARTUZA RL, RABKIN SD, JOHNSON PA. Oncolytic herpes simplex virus vector with enhanced MHC class I presentation and tumor cell killing. Proc Natl Acad Sci USA 2001;98 (11):6396–6401. Engineering of a very promising oncolytic virus for brain tumors; although it has not been tested in clinical trials, preclinical research has demonstrated that this virus has significantly increased both safety and replication capacity when used for brain tumors. [PubMed: 11353831]

92. KO D, HAWKINS L, YU DC. Development of transcriptionally regulated oncolytic adenoviruses. Oncogene 2005;24(52):7763–7774. [PubMed: 16299536]

93••. BISCHOFF JR, KIRN DH, WILLIAMS A, et al. An adenovirus mutant that replicates selectively in p53-deficient human tumor cells. Science 1996;274(5286):373–376. Describes the first oncolytic virus targeting a specific genetic/molecular feature of cancer cells; even though further research has found that the selectivity of this virus for cancer cells is more complex than what was described here, it has opened a new field of research. [PubMed: 8832876]

94. FUEYO J, GOMEZ-MANZANO C, ALEMANY R, et al. A mutant oncolytic adenovirus targeting the Rb pathway produces anti-glioma effect *in vivo*. Oncogene 2000;19(1):2–12. [PubMed: 10644974]

95. GOMEZ-MANZANO C, BALAGUE C, ALEMANY R, et al. A novel E1A–E1B mutant adenovirus induces glioma regression *in vivo*. Oncogene 2004;23(10):1821–1828. [PubMed: 15014451]

96. FARASSATI F, YANG AD, LEE PW. Oncogenes in Ras signalling pathway dictate host-cell permissiveness to herpes simplex virus 1. Nat Cell Biol 2001;3 (8):745–750. [PubMed: 11483960]

97. SHAH AC, PRICE KH, PARKER JN, et al. Serial passage through human glioma xenografts selects for a deltagamma134.5 herpes simplex virus type 1 mutant that exhibits decreased neurotoxicity and prolongs survival of mice with experimental brain tumors. J Virol 2006;80(15):7308–7315. [PubMed: 16840311]

98. BOVIATSIS EJ, PARK JS, SENA-ESTEVES M, et al. Long-term survival of rats harboring brain neoplasms treated with ganciclovir and a herpes simplex virus vector that retains an intact thymidine kinase gene. Cancer Res 1994;54(22):5745–5751. [PubMed: 7954393]

99. CHASE M, CHUNG RY, CHIOCCA EA. An oncolytic viral mutant that delivers the CYP2B1 transgene and augments cyclophosphamide chemotherapy. Nat Biotechnol 1998;16(5):444–448. [PubMed: 9592392]

100. TYMINSKI E, LEROY S, TERADA K, et al. Brain tumor oncolysis with replication-conditional herpes simplex virus type 1 expressing the prodrug-activating genes, CYP2B1 and secreted human intestinal carboxylesterase, in combination with cyclophosphamide and irinotecan. Cancer Res 2005;65(15):6850–6857. [PubMed: 16061668]

101. AGHI M, RABKIN S, MARTUZA RL. Effect of chemotherapy-induced DNA repair on oncolytic herpes simplex viral replication. J Natl Cancer Inst 2006;98 (1):38–50. [PubMed: 16391370]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

102•. LAMPSON LA. New animal models to probe brain tumor biology, therapy, and immunotherapy: advantages and remaining concerns. J Neurooncol 2001;53(3):275–287. Comprehensive review on animal models for brain tumor preclinical research; this review clearly shows the difficulty in translating gene therapy preclinical data from the available animal models to humans. [PubMed: 11718260]

103. BRINSTER RL, CHEN HY, MESSING A, VAN DYKE T, LEVINE AJ, PALMITER RD. Transgenic mice harboring SV40 T-antigen genes develop characteristic brain tumors. Cell 1984;37 (2):367–379. [PubMed: 6327063]

104. CORCORAN RB, SCOTT MP. A mouse model for medulloblastoma and basal cell nevus syndrome. J Neurooncol 2001;53(3):307–318. [PubMed: 11718263]

105. UHRBOM L, HOLLAND EC. Modeling gliomagenesis with somatic cell gene transfer using retroviral vectors. J Neurooncol 2001;53(3):297–305. [PubMed: 11718262]

106. DING H, GUHA A. Mouse astrocytoma models: embryonic stem cell mediated transgenesis. J Neurooncol 2001;53(3):289–296. [PubMed: 11718261]

107. EARNEST FT, KELLY PJ, SCHEITHAUER BW, et al. Cerebral astrocytomas: histopathologic correlation of MR and CT contrast enhancement with stereotactic biopsy. Radiology 1988;166(3): 823–827. [PubMed: 2829270]

108. HERHOLZ K, WIENHARD K, HEISS WD. Validity of PET studies in brain tumors. Cerebrovasc Brain Metab Rev 1990;2(3):240–265. [PubMed: 2121212]

109. PRICE P. PET as a potential tool for imaging molecular mechanisms of oncology in man. Trends Mol Med 2001;7(10):442–446. [PubMed: 11597518]

110•. JACOBS AH, DITTMAR C, WINKELER A, GARLIP G, HEISS WD. Molecular imaging of gliomas. Mol Imaging 2002;1(4):309–335. Comprehensive review on molecular imaging of gliomas; it clearly shows how gene therapy has impacted the development of this field. [PubMed: 12926228]

111. CHO JY, XING S, LIU X, et al. Expression and activity of human Na+/I- symporter in human glioma cells by adenovirus-mediated gene delivery. Gene Ther 2000;7(9):740–749. [PubMed: 10822300]

112. BOLAND A, RICARD M, OPOLON P, et al. Adenovirus-mediated transfer of the thyroid sodium/ iodide symporter gene into tumors for a targeted radiotherapy. Cancer Res 2000;60(13):3484–3492. [PubMed: 10910060]

113. JACOBS A, TJUVAJEV JG, DUBROVIN M, et al. Positron emission tomography-based imaging of transgene expression mediated by replication-conditional, oncolytic herpes simplex virus type 1 mutant vectors *in vivo*. Cancer Res 2001;61(7):2983–2995. [PubMed: 11306477]

114. REHEMTULLA A, HALL DE, STEGMAN LD, et al. Molecular imaging of gene expression and efficacy following adenoviral-mediated brain tumor gene therapy. Mol Imaging 2002;1(1):43–55. [PubMed: 12920860]

115. FLOETH FW, AULICH A, LANGEN KJ, BURGER KJ, BOCK WJ, WEBER F. MR imaging and single-photon emission CT findings after gene therapy for human glioblastoma. Am J Neuroradiol 2001;22(8):1517–1527. [PubMed: 11559499]

116. MORANDI E, ZINGARETTI C, CHIOZZOTTO D, et al. A cDNA-microarray analysis of camptothecin resistance in glioblastoma cell lines. Cancer Lett 2006;231(1):74–86. [PubMed: 16356833]

117. HUSSAIN SF, YANG D, SUKI D, GRIMM E, HEIMBERGER AB. Innate immune functions of microglia isolated from human glioma patients. J Transl Med 2006;4:15. [PubMed: 16573834]

118. CONDEELIS J, POLLARD JW. Macrophages: obligate partners for tumor cell migration, invasion, and metastasis. Cell 2006;124(2):263–266. [PubMed: 16439202]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Table 1

Results from clinical trials with non-replicating viruses.

Reference	Year	Tumor	Vector	Genes	Patients (n)	Primary goal	Survival (months)
[38]	1996	recurrent	RVPC	*HSV-TK*	5	feasibility and safety	ND (1–8)
[39]	1997	recurrent	RVPC	*HSV-TK*	15	feasibility and safety	8 (NR)
[40]	1998	recurrent	RVPC	*HSV-TK*	12	feasibility and safety	6.9 (nd ≥ 33.6)
[41]	1999	recurrent	RVPC	*HSV-TK*	48	feasibility and safety	8.6 (2 ≥ 30)
[42]	2000	recurrent	RVPC	*HSV-TK*	5	efficacy assessment	ND (8.4 ≥ 12)
[43]	2000	recurrent	RVPC	*HSV-TK*	5	toxicity and virus transduction	ND (1 ≥ 18)
[44]	2000	recurrent	RVPC	*HSV-TK*	12	feasibility and safety	NR
[45]	2000	primary	RVPC	*HSV-TK*	248	efficacy gene therapy versus standard therapy	median survival: 12.2 versus 11.8
[46]	1999	recurrent	RVPC	*HSV-TK/IL2*	4	efficacy assessment	NR
[47]	2005	recurrent	RVPC	*HSV-TK/IL2*	12	efficacy assessment	7.5 (1–29)
[48]	2000	both	RVPC and adenovirus	*HSV-TK*	22	compare adenovirus/retrovirus/control	15 (8–21)/7.4 (4–13)/8.3 (3–12)
[49]	2000	recurrent	adenovirus	*HSV-TK*	13	toxicity assessment	8.5 (1.5–36)
[50]	2003	recurrent	adenovirus	*HSV-TK*	11	toxicity assessment	13 (1–26)
[51]	2003	primary	adenovirus	*TP53*	15	virus molecular effects and transduction	9.5 (2.9–39.8)
[52]	2004	both*	adenovirus	*HSV-TK*	36	efficacy of gene therapy versus standard therapy	median survival: 15.7 versus 8.7
[20]	2003	recurrent	liposomes	*HSV-TK*	8	feasibility and safety	7 (NR)

Survival is indicated as: median survival (minimum survival – maximum survival).

*both: primary and recurrent.

HSV-TK: Herpes simplex virus type 1 thymidine kinase; ND: Not determined; NR: Not reported; RVPC: Retrovirus-producing cells.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

Table 2

Results from clinical trials with replication-competent viruses.

Reference	Year	Tumor	Vector	Genetic changes	Patients (n)	Primary goal	Survival (months)
[53]	2000	recurrent	HSV-G207	*UL39*$^{-}$/*ICP34.5*$^{-}$	21	toxicity assessment	6.2 (1 ≥ 19)*
[54]	2000	recurrent	HSV-1716	*ICP34.5*$^{-/-}$	9	toxicity assessment	ND (3–17)
[55]	2004	recurrent	adenovirus-ONYX-015	*E1B-55kD*$^{-/-}$	24	toxicity assessment	6.2 (1.3–28)
[56]	2005	recurrent	NDV-HUJ	none	11	toxicity assessment	7.4 (0.75–14.7)

Survival is indicated as: median survival (minimum survival - maximum survival).

HSV: Herpes simplex virus; ND: Not determined; NDV: Newcastle disease virus.